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Light exposure during storage preserving soluble sugar and L-ascorbic acid content of minimally processed romaine lettuce (*Lactuca sativa* L.var. *longifolia*)

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ABSTRACT

Minimally processed romaine lettuce (MPRL) leaves were stored in light condition (2500 lux) or darkness at 4 °C for 7 d. Light exposure significantly delayed the degradation of chlorophyll and decrease of glucose, reducing sugar, and sucrose content, and thus preserved more total soluble solid (TSS) content at the end of storage in comparison with darkness. While, it did not influenced starch content that progressively decreased over time. The L-ascorbic acid (AA) accumulated in light-stored leaves, but deteriorated in dark-stored leaves during storage. The dehydroascorbic acid (DHA) increased in all leaves stored in both light and dark condition, of which light condition resulted in less DHA than darkness. In addition, the fresh weight loss and dry matter significantly increased and these increases were accelerated by light exposure. Conclusively, light exposure in applied intensity effectively alleviated MPRL quality deterioration by delaying the decreases of pigments, soluble sugar, TSS content and accumulating AA.
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1. Introduction

Romaine lettuce is consumers' favourite leafy vegetable for its crispness, good aroma, tender appearance and high phytochemicals like phenolic compounds (Llorach, Martínez-Sánchez, Tomás-Barberán, Gil, & Ferreres, 2008; Martínez-Sánchez, Tudela, Luna, Allende, & Gil, 2011). Unfortunately, romaine lettuce is naturally perishable and susceptible to quality deterioration after harvest and thus has a short shelf-life. Various chemical treatments such as reducing agents and browning inhibitors have been applied to control tissue browning in order to alleviate quality loss (Roura, Pereyra, & Vallea, 2008; Saltveit, Choi, & Tomás-Barberán, 2005). However, consumers' concerns about chemical toxicity preclude the chemical application. Therefore, it is urgent to seek alternatives to preserve fresh romaine lettuce quality without use of chemicals to meet consumer acceptability.

Light exposure currently represents a novel approach and is widely used to preserve the overall quality of fresh produce (Lester, Makus, & Hodges, 2010; Manzocco, Quarta, & Dri, 2009; Noichinda, Bodhipadma, Mahamontri, Narongruk, & Ketsa, 2007; Toledo, Ueda, Imahori, & Ayaki, 2003; Zhan, Hu, Li, & Pang, 2012a; Zhan, Li, Hu, Pang, & Fan, 2012b). In comparison with traditional preservative methods, light exposure is a non-toxic, cheap, free of residuals, and environmental-friendly treatment (Manzocco et al., 2009). In view of this, extensive studies were carried out to investigate the effect of light exposure at various intensities and photoperiod on quality and physiology of fresh fruits and vegetables during postharvest storage (Büchert, Gómez Lobato, Villarreal, Civello, & MartIneza, 2011; Lester et al., 2010; Martínez-Sánchez et al., 2011; Noichinda et al., 2007; Olarte, Sanz, Echávarri, & Ayala, 2009; Zhan et al., 2012a, 2012b). More recently, we found that intensity of 2500 lux light exposure effectively protected fresh-cut romaine lettuce from browning and quality decay by inhibiting browning-related enzyme activity and maintaining nutritional constituents during refrigeration (Zhan et al., 2012b). Continuous white light illumination during postharvest storage supports photosynthetic capacity of fresh spinach leaves, resulting in increased availability of soluble carbohydrates (Toledo et al., 2003). Like spinach leaves, romaine lettuce leaves are green and tender when harvested and can continuously photosynthesize by which the light energy is converted to chemical energy and stored in the bonds of sugar. Thus, we hypothesise that light exposure during postharvest storage may regulate carbohydrate metabolism of romaine lettuce leaves.

The aim of this study is to determine how continuous light exposure (2500 lux) affects minimally processed romaine lettuce (MPRL) carbohydrate content associated with glucose, reducing sugar, sucrose, starch, and total soluble solid (TSS) content upon 7 d cold storage. In addition, the chlorophyll, L-ascorbic acid (AA), dehydroascorbic acid (DHA), dry matter content, and fresh weight loss were also evaluated during storage, respectively.

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2. Materials and methods

2.1. Sample preparation

Romaine lettuces (*Lactuca sativa* L.var. *longifolia*) were obtained from local farm and brought to laboratory within 2 h for experiment. The plants were selected for uniformity of colour and size. The selected lettuces were washed with cold tap water (5 °C ± 2) for getting rid of soil. Then, the roots, outer old leaves, and core of washed lettuce were removed using sharp stainless steel knife. The remaining leaves were subsequently washed in cold tap water (5 °C ± 2) again before surface-sterilized by immersion in 0.2% (v/v) NaClO solution (15 L/kg, pH 6.5 adjusted with citric acid) for 30 s. The excess surface water on leaves was air-dried at 12 °C for 20 min. After this, about 200 g material for each sample was arranged one layer in 35 cm × 30 cm plastic tray (Botong plastic Co. Ltd, Beijing, China) and wrapped with 35 μm polypropylene film (Tianjin Luda Ltd. Corp., Tianjin, China) with O2 permeability of 6,800 cm^3/m^2 d at 25 °C to minimise desiccation.

All of 18 packaged samples were randomly separated into two bunches. One bunch with nine samples were stored under light (2500 ± 2 lux) condition and the other bunch with nine samples were stored under dark (0.2 lux) conditions at 4 °C for 7 d, respectively. The light illumination was obtained as described in our previous report (Zhan et al., 2012b). Parameters were analyzed at pre-storing (0), 1, and 7 d after storage.

2.2. Pigment content analysis

A 10 g fresh tissue from each sample was blended and 2 g blending paste was extracted in 20 ml 80% acetone/water (v/v) overnight at 4 °C in dark condition. The extract was centrifuged at 3000*g* for 10 min and supernatant was used for the spectrophotometric determination of chlorophyll a (Chl *a*) and chlorophyll b (Chl *b*) at wavelength of 662 and 645 nm, respectively. For each treatment and each period, three samples were used. The amount of these pigments was calculated according to the Lichtentaler and Wellburn method (1983).

2.3. Soluble sugars, starch and TSS content measurement

About 100 g fresh leaves from each sample were dried in an oven (DHG-9053A, Shanghai Heheng Instrument & Equipment Co. Ltd., Shanghai, China) at 65 ± 1 °C until constant weight. The dried leaves were transferred to mortar and ground into powder using as measurement of sugar and starch. For extraction of soluble sugars and starch, accurate 0.050 g dried power was extracted in 10 ml 85% ethanol solution and incubated in water bath at 60 °C for 20 min. Then, the extract solution was centrifuged at 3000*g* for 10 min and the supernatant was collected. The pellet was re-extracted twice with same solvent and the supernatants were combined. The total supernatant was filtered through Whatman filter paper (Grade 1, Hangzhou Whatman-Xinhua filter paper Co., Ltd., Hangzhou, China) after adding activated carbon in order to remove pigments. The filtrate was collected for soluble sugar measurement. The pellet left after extraction of soluble sugars was extracted in 5 ml distiled water and 6.5 ml 52% perchloric acid for determination of starch as proposed by Morris (1948).

Glucose, sucrose, and starch were estimated by anthrone-sulphuric acid method of Yem and Willis (1954) and Morris (1948). Standard curve was plotted with glucose and sucrose, respectively. Reducing sugars were quantified by the 3, 5-dinitrosalicylic acid method at 540 nm wavelength as proposed by Miller (1959) with glucose as standard curve.

TSS content was measured using hand refractometer (Model N1; Atago, Tokyo, Japan). Briefly, 10 g fresh tissue from each sample was ground and the grinding paste was subsequently squeezed through four-layer cotton cloth. A few drops of squeezed juice were dropped onto refractometer window for reading Brix value. The results were expressed in degree Brix.

2.4. AA and DHA content analysis

AA and DHA were determined spectrophotometrically according to the methodology of Kampfenkel, Montagu, and Inzé (1995). A 20 g of fresh material was ground in 40 ml 6% of freezing trichloroacetic acid/water (w/v) on ice. The homogenate was centrifuged at 15,000*g* at 4 °C for 10 min and the resultant supernatant was immediately used for total AA and AA analysis. The DHA content was computed from the difference between the total AA and AA. The results were expressed as milligram per 100 grams of fresh weight (mg/100 g FW) based on the calibrations compared with standard curves produced by freshly prepared L-ascorbic acid and dehydroascorbic acid.

2.5. Fresh weight loss and dry matter content assay

Both fresh weight loss and dry matter content assay were measured according to our previous methodology (Zhan et al., 2012a). The results were expressed as percentage.

2.6. Statistical analysis

All data in triplicates from three samples for each treatment were submitted to the analysis of variance using SPSS 11.0 (SPSS Inc., Chicago, IL, USA). The results were expressed as the means ± SD. One-way ANOVA was applied to compare the effect of light condition on measured parameters using the least significant difference (LSD) test at 0.05 confidence level.

All the extractions were carried out under the lab light condition (approx. 200 lux) with the exception of extra explanation and all the chemicals were analytical reagents purchased from Huafeng chemical reagent Co. Ltd. (Zhengzhou, China) and Sangon Biotech Co. Ltd. (Shanghai, China). And all the spectrophotometric analyses were conducted using Shimadzu spectrophotometer (UV-2401PC, Shimadzu Co. Ltd, Kyoto, Japan).

3. Results and discussion

3.1. Chlorophyll content

Light exposure significantly affected MPRL pigment content that displayed progressive degradation over time regardless of treatments (Fig. 1). Chl *a* content showed significant decrease in all leaves during storage (Fig. 1A). However, compared to dark-stored leaves, light stored leaves preserved remarkably more Chl *a* during storage, implying that light condition was efficient to alleviate Chl *a* degradation. Similar to Chl *a*, Chl *b* content in samples stored under both light and dark condition deteriorated over time, and this deterioration was markedly delayed by light exposure (Fig. 1B). At first and seventh day storage, Chl *b* content in samples exposed to light condition were more 20% and 22% than that in samples stored in darkness, respectively. The total chlorophyll content, sum of Chl *a* and Chl *b*, showed consistent tendency with Chl *a* during storage (Fig. 1C).

Yellowing of green leafy vegetables upon storage is normally considered to be the major consequences of chlorophyll degradation (Brown, Houghton, & Hendry, 1991). In the present study, MPRL leaves de-greened progressively with the chlorophyll

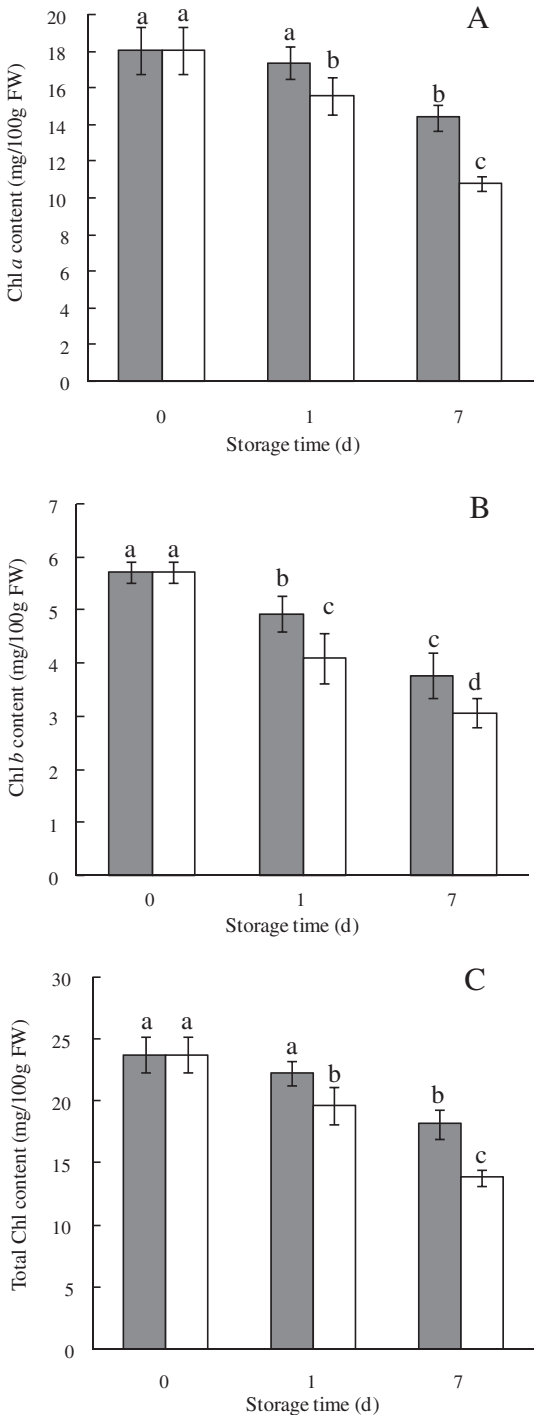

Fig. 1. Effects of light exposure on chlorophyll a (*Chl a*) (A), chlorophyll (Chl *b*) (B), and total Chlorophyll (total Chl) (C) content of MPRL stored at 4 °C for 7 d. Each value is the mean of triplicates from three independent samples ± SD. Different letters indicate significant differences between treatments during storage ($P < 0.05$). ■, 2500 lux light exposure. □, darkness.

deterioration during storage. However, such undesirable degradation was significantly delayed by light exposure, indicating that the light exposure could be beneficial to maintenance of MPRL green colour. Possible reason may be that the fresh green vegetables still maintained photosynthetic activity and synthesized photosynthetic substances under light condition during initial storage, which probably contributed to delaying tissue deterioration (Büchert et al., 2011; Zhan et al., 2012a). Similar result was also reported in Chinese kale leaves, of which chlorophyll degradation was alleviated by light exposure during storage (Noichinda et al., 2007). In addition, the current data indicated that Chl *b* degraded more dramatically than Chl *a* in both light (decreased by 34% and 20%, respectively) and darkness (decreased by 46% and 40%, respectively). These data was in accordance with the model that the first step of Chl *b* degradation apparently requires conversion to Chl *a* (Noichinda et al., 2007; Scheumann, Schoch, & Rüdiger, 1999; Tanaka, Tanaka, Takabe, & Tsuji, 1995), resulting the degradation of Chl *a* was impeded.

3.2. Soluble sugars, starch and TSS content

As expected, in comparison with darkness, light exposure markedly preserved the glucose, reducing sugar, and sucrose content that significantly decreased over time (Fig. 2). The glucose concentration decreased slower in leaves stored in light (decreased by 33%) than those in leaves stored in dark (decreased by 42%) at the end of storage (Fig. 2A). Similarly, reducing sugar content reduced by 42% and 47% in leaves exposed light and dark conditions after 7 d storage, respectively (Fig. 2B). And sucrose followed the same trend as glucose and reducing sugar for all leaves although it dropped mildly over time, which only decreased by 8% and 12% in light and dark conditions at the end of storage, respectively (Fig. 2C). Unlike soluble sugars, starch content was not significantly influenced by light exposure although its content showed markedly dropped over time (Fig. 3A). At each storage day, the starch level in leaves stored under both light and dark condition was approximately same.

As known, sugars, especially glucose, are the direct products of photosynthesis in green plant tissue when exposed to light. And at the same time, they are also the primary substrates of respiration. Thus the actual assayed concentrations of sugars in plant tissue rely on the balance between their synthesis and consumption. Current data showed that light exposure during storage significantly preserved MPRL soluble sugar content over time compared to dark condition. The higher sugar content might result from the continuous photosynthesis of leaves when exposed to light condition as continuous light illumination supports the photosynthetic capacity of green leaves during storage (Toledo et al., 2003). Similar results were also found in fresh spinach and Chinese kale leaves in which the soluble carbohydrates and monosaccharides such as glucose increased when stored under light illumination condition (Noichinda et al., 2007; Toledo et al., 2003). In spite of this, the starch, as storage sink of glucose, was not influenced by light exposure over time, indicating MPRL leaves exposed to light did not accumulate enough glucose to convert into starch during such short storage period. This was not in agreement with Chinese kale leaves that displayed an increase in starch content besides glucose and fructose when exposed to light storage condition (Noichinda et al., 2007). Such differences might come from the various materials, light conditions, processing operations, and storage period.

Very similar to tendency of sugars, the TSS in MPRL exhibited significant decrease at the end of storage regardless of treatments (Fig. 3B). However, light exposure obviously delayed this decrease at the last day of storage compared to darkness. Light-stored samples contained more of 5.1% TSS than those stored in darkness at 7 d storage. It is speculated the higher TSS content mainly resulted from the higher soluble sugar content as these sugars are the main contributors of TSS in plant tissue.

3.3. AA and DHA content

During the whole postharvest storage, MPRL leaves exposed to light revealed noticeable high AA in comparison with those stored in the dark (Fig. 4A). In light-stored leaves, AA content almost

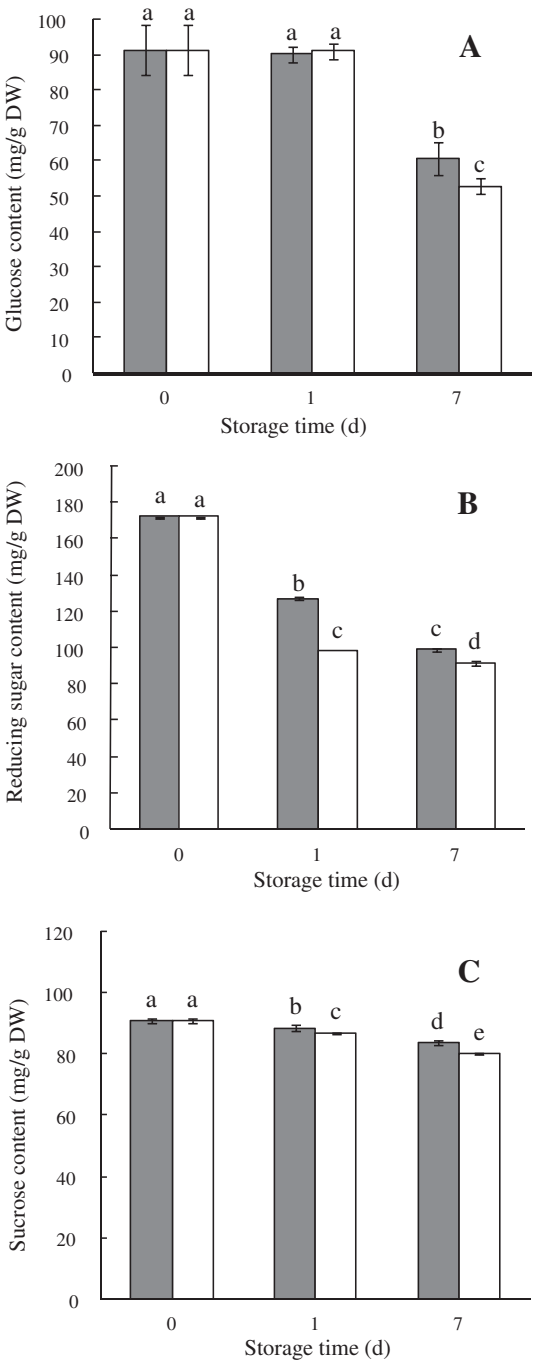

Fig. 2. Effects of light exposure on glucose (A), reducing sugar (B), and sucrose (C), content of MPRL stored at 4 °C for 7 d. Each value is the mean of triplicates from three independent samples ± SD. Different letters indicate significant differences between treatments during storage ($P < 0.05$). ■, 2500 lux light exposure. □, darkness.

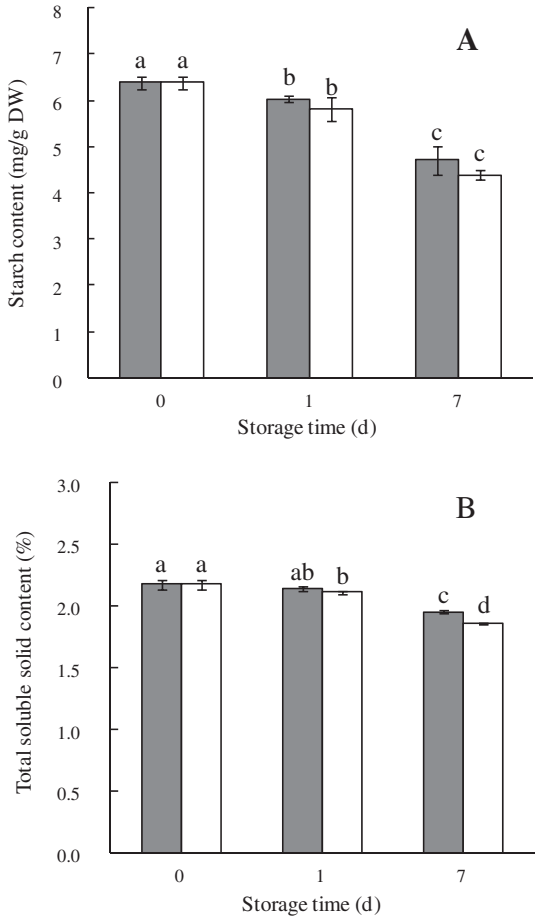

Fig. 3. Effects of light exposure on starch (A) and total soluble solids content (B) content of MPRL stored at 4 °C for 7 d. Each value is the mean of of triplicates from three independent samples ± SD. Different letters indicate significant differences between treatments during storage ($P < 0.05$). ■, 2500 lux light exposure. □, darkness.

increased by 23% from the initial level of 7.1 mg/100 g FW at first day storage and then fell back to the pre-storage level after 7 d storage. On the contrary, in dark-stored leaves, AA significantly deteriorated over time and dropped by 8.2% and 28.9% at first day and seventh day storage compared to the pre-storage level, respectively. Overall, MPRL leaves exposed to light contained more of 34% and 45% AA than those stored in darkness at first day and seventh day storage, respectively. This result was in accordance with our recent findings that verified the high intensity light maintained or slight decreased AA content, while dark storage condition resulted in 40.3% AA deterioration of fresh-cut romaine lettuce leaves after 7 d storage at 4 °C (Zhan et al., 2012b).

L-ascorbic acid abundantly exists in many horticultural crops and is affected by a wide range of factors such as genotype, cultivate factors, and pre- or post-harvest conditions (Lee & Kader, 2000). According to the literatures, AA is synthesized via a sequence of hexose precursors that primarily involved D-glucose (Loewus, Jang, & Seegmiller, 1956). One pathway of AA synthesis involves the conversion of glucose to D-glucosone, then to L-sorbosone and finally to AA, although the metabolic pathway of AA biosynthesis in high plants has not been conclusively established (Toledo et al., 2003). Therefore, it was suggested that the higher hexose, especially glucose, content was responsible for higher AA content in light-stored leaves. The increased availability of these sugars could contribute to the control of AA pool size (Toledo et al., 2003). Such relationship between AA and soluble carbohydrate content has been also observed in fresh leaves of spinach and Chinese kale during light condition storage (Noichinda et al., 2007; Toledo et al., 2003).

DHA progressively accumulated over time regardless of the treatment (Fig. 4B). In light-stored leaves, DHA accumulated slowly and was nearly 1.7-fold of the initial value (1.8 mg/100 FW) after 7 d storage. However, in dark-stored leaves, DHA increased sharply over time and was 2.5-fold of the initial level at the end of storage. DHA is the oxidised form of AA and the conversion from AA to DHA is catalysed by some enzymes such as ascorbate peroxidase and

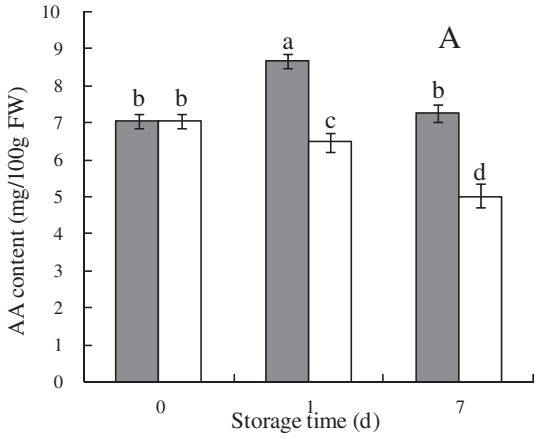

Fig. 4. Effects of light exposure on ascorbic acid (AA) (A) and dehydroascorbic acid (DHA) (B) content of MPRL stored at 4 °C for 7 d. Each value is the mean of triplicates from three independent samples ± SD. Different letters indicate significant differences between treatments during storage ($P < 0.05$). ■, 2500 lux light exposure. □, darkness.

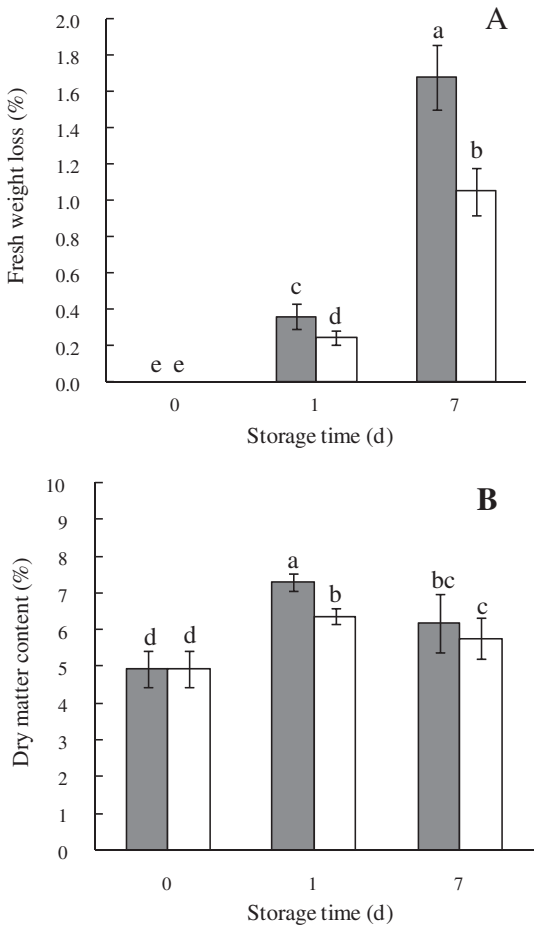

Fig. 5. Effects of light exposure on fresh weight loss (A) and dry matter content (B) of MPRL stored at 4 °C for 7 d. Each value is the mean of triplicates from three independent samples ± SD. Different letters indicate significant differences between treatments during storage ($P < 0.05$). ■, 2500 lux light exposure. □, darkness.

ascorbate oxidase. The opposite direction of this reaction, which would regenerate AA, is not thermodynamically the most favored. Thus, AA tended to decrease, and meanwhile DHA increased during storage of fresh-cut produce (Agar, Massantini, Hess-Pierce, & Kader, 1999). According to the current result, light exposure seemed to be beneficial to alleviate the conversion of from AA to DHA, resulting to less DHA and more AA content in light-stored leaves than dark-stored leaves.

3.4. Fresh weight loss and dry matter content

The fresh weight loss rapidly increased over time and was significantly influenced by storage conditions (Fig. 5A). Light exposure obviously accelerated fresh weight loss over time in comparison with darkness. After 7 d storage, light and dark storage condition resulted in 1.68% and 1.10% fresh weight loss, respectively. In spite of this, all leaves were still turgid and marketable as their fresh weight loss was lower than 2%, the threshold for rendering the leafy greens' texture unacceptable (Wiley, 1994). This was totally in agreement with our previous findings that fresh-cut romaine lettuce reduced 1.74%, 1.35% and 1.05% fresh weight loss when exposed high intensity light, low intensity light, and dark conditions after 7 d storage, respectively (Zhan et al., 2012b). Very similar results were also reported by Martínez-Sánchez et al. (2011) who stated fresh weight loss of fresh-cut romaine lettuce with 0.8%, 0.7%, and 0.4% stored in light, photoperiod, and darkness after 5 d storage, respectively. Differing from fruits or fruit vegetables, leafy vegetables during postharvest storage lose the fresh weight mainly due to transpiration as they generally have a large surface to volume ratio, which makes them vulnerable to rapid water loss after harvest (Kays, 1991). Transpiration regulation through the stomata is well understood in the case of leaves (Ben-Yehoshua & Rodov, 2003). Light exposure has been demonstrated to stimulated stomata opening and moreover the numbers of open stomata have been well correlated with the fresh weight loss of several vegetables (Martínez-Sánchez et al., 2011; Noichinda et al., 2007; Sanz, Olarte, Echávarri, & Ayala, 2007). Therefore, in this case, the fresh weight loss was probably mainly resulted from the stomata open stimulated by light.

Dry matter in fruits and vegetables are the sum of all substances besides of water. As Fig. 5B shown, dry matter content was significantly influenced by storage conditions, of which light exposure resulted in more of 15% dry matter content at first day storage in comparison with darkness. This was confirmed by the data that showed light exposure resulted in more of pigments, soluble sugars, TSS, and AA as these substances are the main components of dry matter. Theoretically, dry matter content in fruits and vegetables tended to decrease during storage owing to the consumption of respiration. In this study, the rapid water loss might contribute to the increase of dry matter content.

In conclusion, intensity of 2500 lux light exposure effectively delayed the decline of MPRL chlorophyll, soluble sugar, and TSS content, and meanwhile increased reduced AA and dry matter

content in comparison with darkness during 7 d storage at 4 °C. Therefore, it could be advisably considered an innovative and economic technique for industry to preserve fresh romaine lettuce. Nevertheless, light exposure accelerated fresh weight loss during storage. To eliminate such undesirable effect, we recommend that light exposure should be applied in combination with other methods such as strictly maintaining cold chain throughout storage, transport, distribution, and sale of MPRL and increasing relative humidity in environment around the products.

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